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FURTHER DATA ON THE INHERITANCE OF BLUE IN POULTRY[1]

PROFESSOR WILLIAM A. LIPPINCOTT

KANSAS AGRICULTURAL EXPERIMENT STATION, MANHATTAN, KANSAS

I. PREVIOUS WORK

The principal facts concerning the genetic behavior of blue in the Andalusian breed of domestic fowl were presented in an earlier paper (Lippincott, 1918a). Previous work on the genetics of the blue Andalusian was reviewed and a limited number of further data were offered.

The latter showed that blue Andalusians are like black Andalusians in that they are self-colored. They are, on the other hand, like the blue-splashed Andalusians in that homologous pigmented feathers in both sexes have the same condition with reference to the restriction of pigment in the feather structure. The $1:2:1$ ratio obtained from mating blue Andalusians together may be interpreted as the combination of two 3 to 1 ratios. These relationships are shown in Fig. 1.

The restriction of black pigment in the feather structure to give the blue appearance found in blue and in blue-splashed Andalusians was shown to be due to the action of a dominant factor R. The extension of black pigment to all feathers of the body as in both black and blue Andalusians, was found to be due to the action of another dominant factor E.
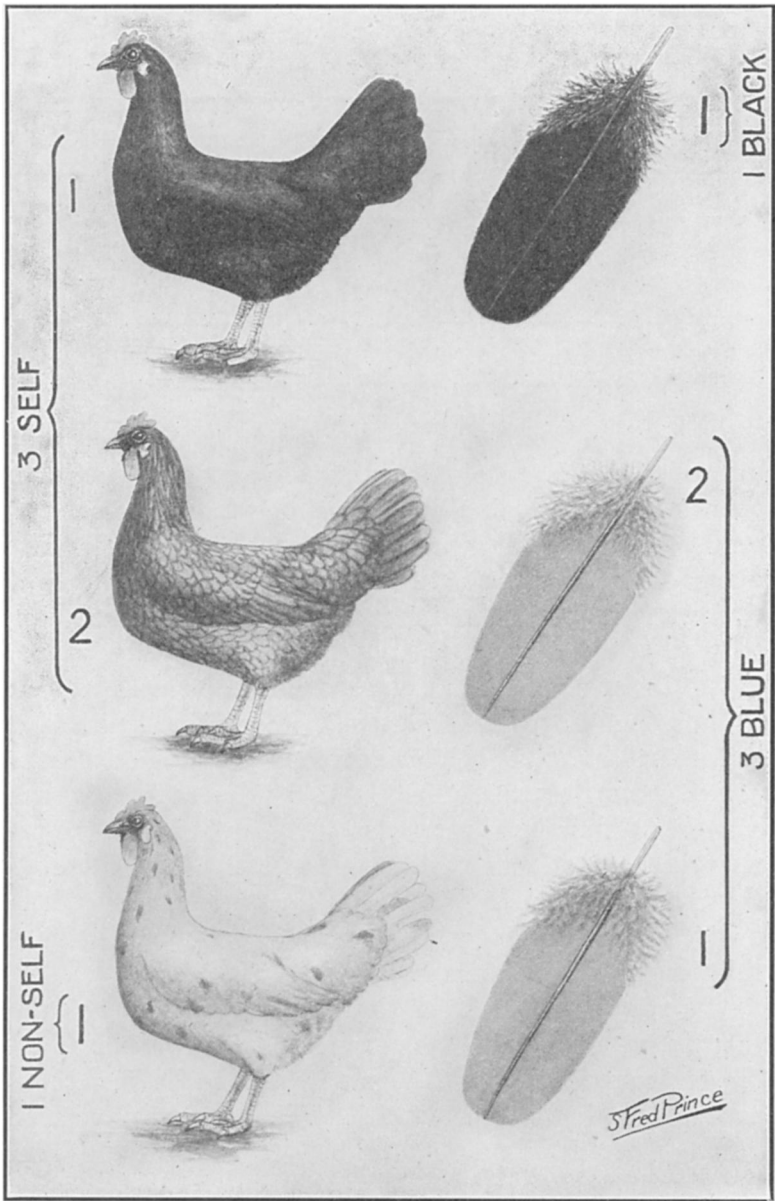
Fig. 1.   Showing that the 1:2:1 ratio is a combination of two 3:1 ratios.

It was pointed out that while, on the basis of their expression in the phenotype it appeared more logical to consider these factors as dominants, each closely linked to the recessive allelomorph of the other, they may, so far as the experimental evidence shows, be considered as true allelomorphs occupying identical loci on homologous chromosomes, and each expressing itself independently of the other.

The finding of crossovers between R and E would be conclusive evidence proving the former of the two conditions proposed. It was shown that while no crossovers had been reported the critical data on the case were very limited and the likelihood of crossovers being detected and isolated by breeders is very small. It might well have been suggested further that even though crossing-over does rarely occur, for instance, so that less than one per cent. of the individuals are the product of crossover gametes, the chances of detecting them experimentally are small, considering the limited number of matings (as determined by the equipment available at most experimental institutions) which are likely to be devoted to a search for crossovers.

Though much has been made of the blue Andalusians as a "heterozygote phenotypically intermediate between the parental types" it was shown that while all self-blues so far found had proved to be heterozygous for R and E, they were not in the strict sense intermediate between the parental types. The F_1 progeny of a cross between blue-splashed Andalusians and white Wyandottes was reported as self-blue and far darker than either parent.

It was further shown in the earlier paper that R not only restricts black pigment, so as to render pigmented areas bluish-gray in appearance, but also affects the shape of the pigment granules, so that instead of appearing as rods as in black individuals, they are quite round. In this particular R is quite dominant over its allelomorph, whether one chooses to assume that the latter is E or r.
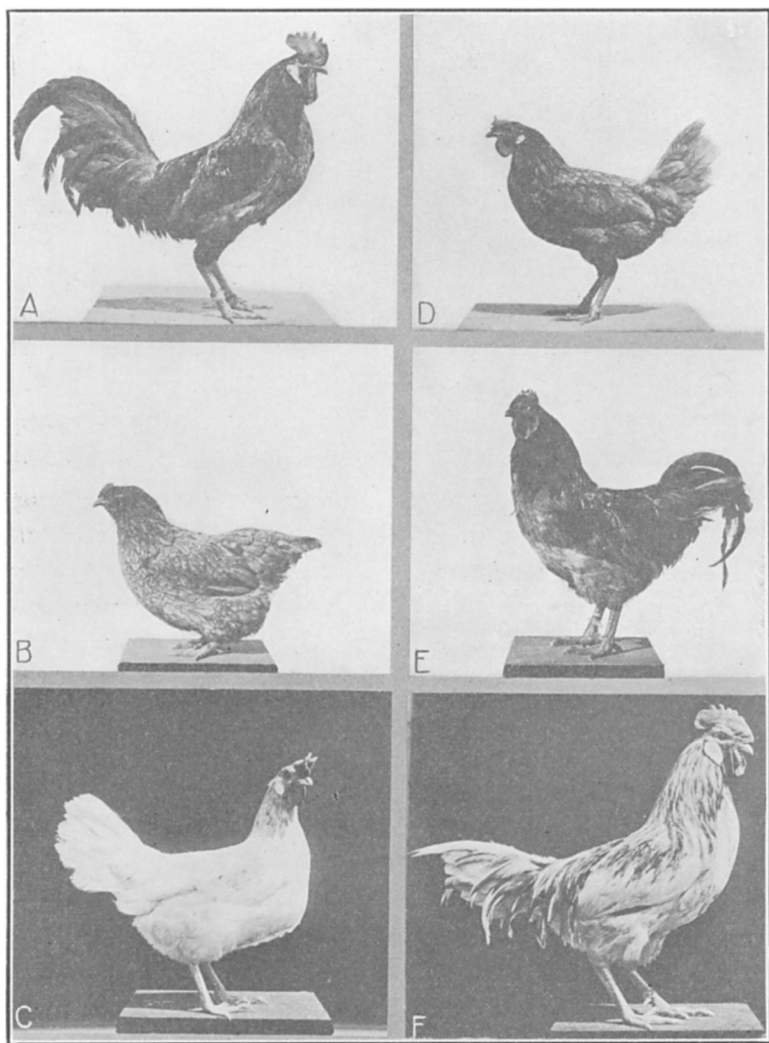
EXPLANATION OF PLATE I

The photographs shown in Plates I and II were taken by James Machir, my indebtedness to whom it is a pleasure to acknowledge.

Fig. A. Blue Andalusian male.
Fig. B. Blue Orpington female.
Fig. C. Blue-splashed Andalusian female.
Fig. D. Blue Andalusian female.
Fig. E. Blue Orpington male.

Fig. F. Blue-splashed Andalusian male.
A and D—Blue Andalusian.
B and E—Blue Orpington.
C and F—Splashed Andalusian.

It was also shown that both the restricting and the rounding actions of R were interfered with in certain regions of both blue-splashed and blue males. In both color types the pigmented feathers of the neck (hackle), back, and saddle are black or bluish-black instead of blue as on the remainder of the body. The black pigment granules in these regions are for the most part rod-shaped rather than round. It was suggested that this interference with the action of R is a secondary sexual characteristic, presumably due to the presence of testicular or the absence of ovarian influence.

II. Purpose of the Present Paper

It is the purpose of this paper to present further data concerning the inheritance of blue and its relations to the sex glands, and to draw such conclusions as these data justify. A report is given of the breeding behavior of blue as found in the Andalusian, Orpington and Leghorn breeds, and of certain crosses of these breeds with each other and with other breeds, which do not possess blue varieties. The relations of the factors involved to certain factors present in the non-blue varieties of other breeds is considered and evidence concerning the relation of the sex-glands to the action of the factor R presented.

III. Material and Methods

The breeding stock used was from several sources, being in part from the pedigreed flock of the University of Wisconsin, where the work reported in the earlier paper was done. It was also in part from the pedigreed flock of Kansas State Agricultural College where the investigation was continued under the direction of Dr. Leon J. Cole of the University of Wisconsin, my indebtedness to whom it is a pleasure to acknowledge. The stock was, however, mostly from unpedigreed lines, though pure-bred within the meaning of the poultryman. In no case were individuals used which were not from families show-
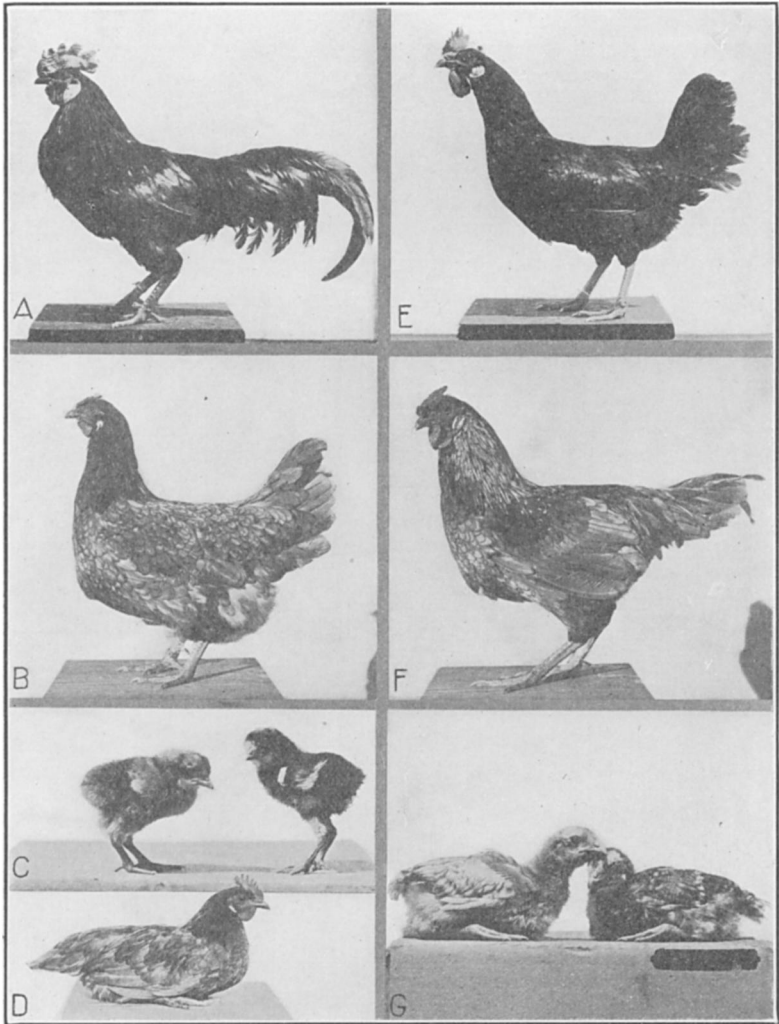
EXPLANATION OF PLATE II

FIG. *A*.   A black Andalusian male.

FIG. *B*.   A blue F_1 female from a white Wyandotte × blue-splashed Andalusian cross.

FIG. *C*.   A blue (at left) and a black (at right) chick in the down.   These are offspring of a white Plymouth Rock ♂ × blue Andalusian ♀.   The occipital spots inherited from the sire are plainly visible.

FIG. *D*.   A young blue F_1 male from a blue-splashed × black Langshan cross.

FIG. *E*.   A black Andalusian female.

FIG. *F*.   A blue F_1 male from a white Wyandotte × blue-splashed Andalusian

ing the characteristics of their respective varieties with constancy in so far as could be learned.   In as much as only varietal (color), as opposed to breed (shape) characteristics were being studied, less attention was paid to the latter in selecting material.   In no case, however, were individuals used which showed disqualifying breed characteristics.

With a single exception no individual was used whose genotype proved to be inconsistent with the ''breeding true'' of the variety to which it belonged, or, in the case of the blue-splashed Andalusian, the variety from which it arose.   This single exception was a blue-splashed Andalusian female (2107) purchased from a breeder who made only blue $\times$ blue matings.   She proved to be heterozygous for P, a factor necessary for the production of black pigment.   The family from which she arose must have been producing occasional whites which were, in all likelihood, being discarded as extremely light blue-splashed wasters from the blue $\times$ blue matings.   This point was not followed up, however, and the facts ascertained.   It has been by taking advantage of situations similar to this one that white varieties have been established in several breeds.

There were several individuals discovered whose factorial composition varied from the normal, or usual, for the varieties to which they respectively belonged.   Owing to the particular factorial complex of which they were a part, however, these factors behaved as cryptomeres, not affecting the adult phenotype of the variety.   Specific reference is made to these individuals in a later section of this paper.

The matings were, for the most part, made in covered

cross.   Indications of a factor or factors for lacing may be seen in the hackle and saddle feathers.

FIG. G.   A blue-barred (at left) and a black-barred (at right) chick partly feathered.   These are offspring of the same mating as the chicks shown in Fig. C this plate.   The barring was inherited from their white Plymouth Rock sire.

A and E—Black Andalusian.

B and F—Blue F_1's.
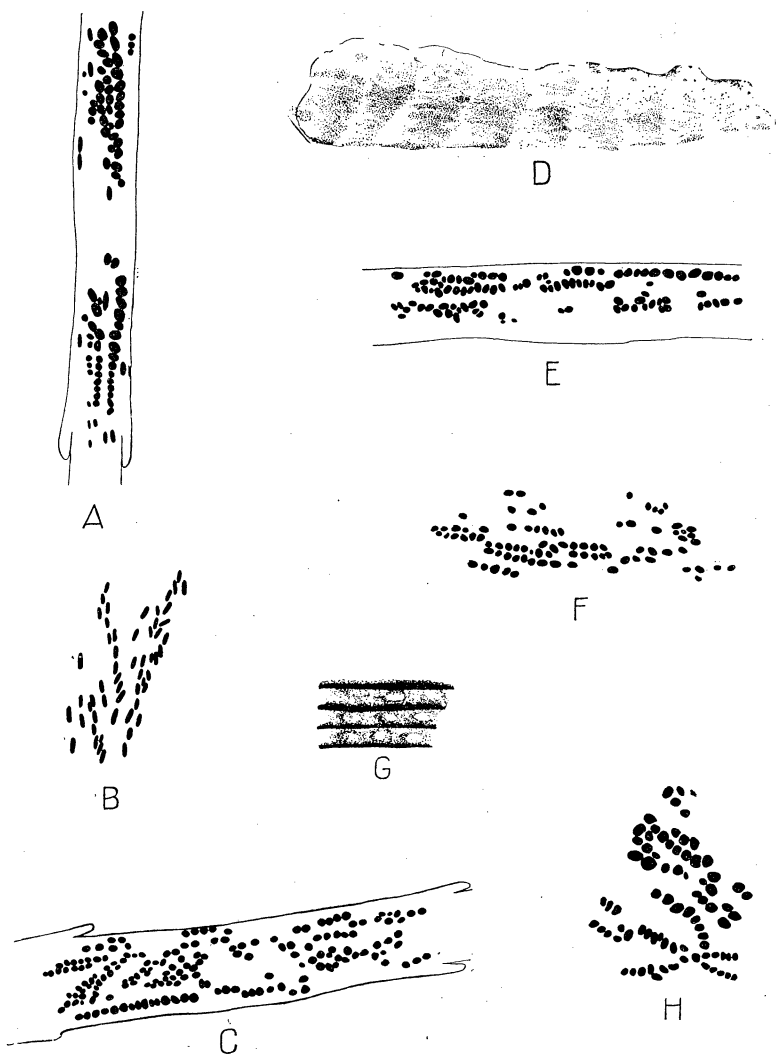
EXPLANATION OF PLATE III

Fig. *A*.   Appearance of pigment granules in a strand of down from a very dark blue crossbred chick.   The sire was a blue F_1 from a white Wyandotte × blue-splashed Andalusian cross.   The mother was a blue Andalusian.   There are occasional rod-shaped granules.   Camera lucida drawing.

Fig. *B*.   Appearance of pigment granules in a.definitive feather from a black Andalusian.   Granules of the same shape are found in the down and definitive feathers of black Langshans, black Orpingtons and the black offspring from crosses with the several breeds used in this investigation.   Camera lucida drawing.

Fig. *C*.   Appearance of pigment granules in a strand of down from a blue

yards and every precaution taken to insure no mixing of matings and the proper identification of the eggs laid by each female in each mating. Not only was the assistant in charge of the trapnesting selected because of his habitual accuracy in details, but the eggs from each individual hen were kept together, separate from the eggs of other individuals, and carefully compared one with another before being put into the incubator. Any off type or off colored eggs were discarded, so far as these experiments are concerned. In spite of these precautions it is too much to hope that some errors have not crept in, though it is believed they are very few.

Owing to the fact that the original stock was of relatively unknown composition, it was necessary to make such matings as would not only throw light on the behavior of the factors under observation, but would also be likely to bring to light unsuspected factors whose action might interefere with the action of the genes being studied. This necessitated introducing test females in the matings where the males were uncertain, and of mating many of the females with test males a second season instead of repeating the mating already made. In both cases the result was to reduce greatly the numbers of offspring from some of the crucial types of matings, and considerable numbers of "test" offspring were hatched and described, for the reporting of which here there is no particular object.

The counts of living chicks were made in the down at hatching time and individual descriptions recorded, each

F_1 chick from a white Wyandotte × blue-splashed Andalusian cross. Camera lucida drawing.

Fig. D. Clumped appearance of pigment granules in a curved barbule from a definitive feather of a blue Andalusian.

Fig. E. Appearance of pigment granules in a strand of down from a blue F_1 chick from a blue-splashed Andalusian × white Plymouth Rock cross. Camera lucida drawing.

Fig. F. Appearance of pigment granules in a definitive feather from a blue Andalusian. Camera lucida drawing.

Fig. G. A small area of the web of a definitive feather from a black Andalusian. The cell boundaries and nuclei may be made out. There is no clumping of pigment within the cells.

Fig. H. Appearance of pigment granules in a definitive feather from a blue Andalusian. Camera lucida drawing.

chick being marked with a numbered wingband. The system of keeping pedigree records in use has been described elsewhere (Lippincott, 1918*b*) and need not be repeated. The descriptions were checked when the chicks were three weeks old, and again at some considerably later, though not specified, time, when the birds were legbanded for the breeding-pen or laying-house, or were sent to market. The descriptions of all chicks dying were carefully checked at the time they were found, though a small number disappeared without their descriptions being checked. Unless there was reason to suspect that their classification might be likely to change after the taking of the first description such individuals were counted.

Fortunately the different classes of offspring could for the most part be distinguished in the down, and counts were accordingly made of chicks which reached an advanced stage of development but which failed to hatch. It was the practise to test all eggs for live germs at the end of the tenth day of incubation and remove all infertile and dead eggs. A second testing was made on the eighteenth day when all the dead eggs found were opened, the embryos described and their sex recorded. On the twenty-second day, after the hatch was well over, all the eggs which failed to hatch were opened and the descriptions of the dead chicks made a matter of record.

In most cases the embryos from crosses among the three color types of Andalusians which passed the first test, developed far enough so that the differentiation between color types could be made with precision after the down had been carefully washed, and dried with the aid of an electric fan. In those crosses involving recessive white parents, only those unhatched chicks could be counted which lived past the eighteenth day.

There were two possible sources of confusion in the classification of the chicks in the down. These were the differentiations between blacks and occasional very dark blues, and between blue-splashed and recessive whites.

The blacks and dark blues could quite readily be separated by examining the down of each chick microscopically. The blacks carry only rod-shaped pigment granules while in dark blue down rounded granules predominate. These are frequently arranged in rows as reported in my former paper (1918a, pp. 98, 99). In the case of every mating where this method of classification was brought into use to aid in distinguishing individuals which failed to hatch, down samples were saved at hatching time from the living dark blue and black chicks as well, and the first description, the record of the microscopic examination, and the later descriptions after definitive feathers were developed were carefully compared and checked. In nearly all cases the descriptions in the down and in the definitive feathers agreed.

In certain matings, however, it was found that descriptions in the down were not reliable and could not be counted. This was particularly true of one family of Andalusians which carried considerable red in the plumage and which has been referred to by Platt (1916) and Pearl (1917) in other connections. Within this family and its crosses the expression of the R factor in the heterozygote was frequently delayed so that individuals which were described in the down as blacks and which showed only rods under the microscope turned out to be blues when the definitive feathers appeared, and then showed the characteristic round pigment granules of the blue. None of the chicks tracing their ancestry to this family are included in the counts herein reported.

The possible source of confusion in the classification in the down of the blue-splashed and the white chicks arises from the fact that while the adults of the white Plymouth Rocks and white Wyandottes are pure white, or very slightly flecked with black, the chicks frequently carry considerable, though varying amounts of black pigment in the down, which gives certain regions a bluish appearance. This varies in degree from near black to slightly

smoky white. Fortunately for the problem in hand the localization of this pigment in the down of certain regions of the body is quite characteristic and quickly recognized. While a blue-splashed chick is frequently very light blue, as noted by Bateson and Punnett (1906, p. 20), the pigment is not localized on the top and back of the head, the wings in the region of the bow, and on the thighs, as it is on the potentially white chick, and the impression conveyed is very different. In potentially white chicks the remiges, which may be seen just starting to grow out from their follicles, are pinkish white and exhibit not the slightest trace of pigment. In the same feathers of the blue-splashed chick, on the other hand, there is a very noticeable bluish cast and usually at least one remex that is distinctly pigmented.

Though in pure-bred white Plymouth Rock and white Wyandotte chicks the pigment granules in the down are typically rod-shaped this fact is not of assistance in classifying with respect to white and blue-splashed offspring from crosses involving the factor R, since under its influence black pigment granules are round whether in a potentially white or a blue-splashed chick.

Not all chicks from pure-bred white Plymouth Rock and white Wyandotte matings exhibit this juvenile pigment. Some can only be recorded as white. It is of interest that the only chicks, three in number, which were originally described as "white, no pigment" or "creamy white" and later used in a breeding pen, have all proved to carry a factor for dominant white, as described in a later section of this paper. The number of such birds which have been tested is small and no general conclusions can be drawn, but the results are suggestive. It is rather interesting to note that a photograph of a group of white Plymouth Rock chicks in "The Plymouth Rock Standard and Breed Book" (American Poultry Association, 1919, p. 419), which is the official guide for the breeding and judging of all Plymouth Rocks, shows individuals

which are noticeably pigmented. In response to a letter of inquiry Professor Arthur Smith of the University of Minnesota, the editor of this book, tells me that my observation concerning the presence of pigment in these chicks is correct and he adds in substance that the pigmented chicks develop into the whitest adults.

The fertility and hatching power of the eggs from the various crosses here reported and the viability of the chicks hatched was increasingly disappointing from season to season. While the comparative coefficients of fertility and hatching power have not been calculated, the ratio between the eggs set and chicks hatched has undoubtedly been lower on the average, than for the purebred unrelated matings of the same and other breeds, set in the same incubator at the same time, and certainly lower than would be counted satisfactory in ordinary poultry husbandry practise.

The foregoing applies as well to the rate of mortality. As representative of the numbers surviving to grow definitive feathers in comparison with the counts recorded in the various tables, those of the F_2 from the blue-splashed Andalusian $\male$ × white Wyandotte $\female$ may be given. The counts made when the chicks were feathered were 47 blue, 18 blue-splashed, 37 black, and 42 white. The total count recorded (see Table IV, group 1) was 100 blue, 46 blue-splashed, 65 black, and 64 white. The reasons for the low hatchability and high mortality have not been established.

Until considerably more data than are now available have been secured it seems best to call attention to the possibility of crossing-over between the loci of R and E by indicating their possible recessive allelomorphs. It is accordingly the practise in this paper to indicate these factors thus: (Re) and (rE).

IV. The Relation of Phenotype to Sex

It is convenient to consider the relation of phenotype to sex before examining the progenies of the various

matings. In order to secure evidence concerning this relation, six blue Andalusian males were caponized during the summer of 1919. Into the body cavities of three of them ovarian tissue from nearly related females was introduced, the other three being kept as checks. The operating was done on July 24 and the birds turned out on range with hundreds of other birds one week later. On September 19 one of them (wingband 1387) was killed by a skunk. At that time it was entirely blue, there being less contrast between the regions that are dark in the male (hackle, back and saddle) and the other regions of the body than frequently appears in blue pullets before comb development indicates the approach of the first laying cycle, and indeed in many mature females. Although it was over four months old (hatching date, May 6, 1919) it appeared so much like an immature pullet that it was mistaken for one by the poultryman in charge and by the writer, until its record and description were consulted. Concerning the latter there did not seem to be any chance of error, since the scar made in opening the body cavity was plainly visible.

Such a situation indicates a fairly complete molt between July 24 and September 19. This is not surprising, however, since Rice, Nixon and Rogers (1908, p. 66) have shown that "from the incubator to the laying period the chicks experienced at least four molts, either partial or complete," and it is further well known that a close relation exists between molting and ovarian activity.

The other birds operated on at the same time were at once looked up and described. One of them (wingband 1855) was found to be somewhat intermediate in condition, some of the feathers of the neck and saddle being blue, but somewhat darker in shade than the normally (in the male) blue regions of the body. There were, however, a few scattered feathers which were almost black from the tip halfway down the web toward the fluff. About midway between the tip of the feather and the be-

ginning of the fluff there was a distinct line of demarcation where the black or near-black became a distinct blue. This chick was hatched a little over two weeks later (May 22) than 1387 and had apparently not gone through a complete molt, some feathers in process of growth at the time of the operation and showing ovarian influence on the last regions to develop still remaining.

On October 26 this bird was killed, apparently by a rat, at which time all of the feathers of the neck and saddle regions were distinctly blue, though considerably darker than other parts of the body. The shape of the feathers was characteristically female.

The third male into which ovarian tissue was introduced (wingband 1480) showed no influence of the introduced tissue on September 19. This condition still prevailed when it was sent to market October 26. It appeared normal for a blue capon of that age, over five months, the hackle and saddle being very dark and characteristically male in shape. Presumably the ovarian tissue introduced atrophied without having any effect.

Of the cockerels which were caponized, but had no ovarian tissue introduced, one (wingband 1859) died soon after the operation. The other two (wingbands 1415 and 1492) showed and continued to show typical blue capon characteristics with regard to the color and shape of the saddle and hackle feathers. The feathers were fully as dark as in normal males of the same age, and as they matured were even longer than their homologs in normal males. This result is precisely the same as that observed by the writer several times in blue capons, concerning which no descriptive records were kept.

In this connection it should be observed that in the family of Andalusians here dealt with, it has been not infrequently noticed that certain nearly grown pullets whose combs have not begun to develop, show only very dark feathers in the regions of the neck and back. These same birds after their combs begin to redden, thereby

indicating ovarian activity and the onset of laying, appear to pass through a molt or partial molt whereby the dark feathers of the back region particularly, are gradually replaced by those of a clearer blue. The necks of such females usually remain dark, showing considerable contrast with the other regions of the body, though being by no means as dark as the same region of the blue male.

Although the number of desexed males into which ovaries were introduced was small, it seems fair to conclude in the light of the evidence concerning testicular (Goodale, 1916) and ovarian (Goodale, 1918; Cole and Lippincott, 1919) influence in fowls that the failure of the factor R to express itself as fully in the neck, back and saddle regions of the blue and blue-splashed males as in the females is due to the lack of some necessary cooperative action on the part of the ovary, and not to any inhibitive action on the part of the testis.

V. The Breeding Behavior of Andalusians

New data concerning the breeding behavior of the three color types of Andalusians, as shown by several types of matings, are presented in Table I.

TABLE I

SHOWING THE NUMBERS AND COLOR TYPES OF PROGENIES FROM VARIOUS ANDALUSIAN CROSSES[2]

Group	♂ ♂	♀ ♀		Blue-spl. $(Re) (Re)$	Blue $(Re)(rE)$	Black $(rE) (rE)$
1..	Blue $(Re) (rE)$	× blue $(Re) (rE)$	Obtained... Theoretical.	46 53.5	104 107	64 53.5
2..	Blue $(Re) (rE)$	× black $(rE) (rE)$	Obtained... Theoretical.	00 00	25 24.5	24 24.5
3..	Black $(rE) (rE)$	× blue $(Re) (rE)$	Obtained... Theoretical.	·00· 00	113 101.5	90 101.5
4..	Blue $(Re) (rE)$	× blue-splashed $(Re) (Re)$	Obtained... Theoretical.	1 1	1 1	0 0
5..	Blue-splashed $(Re) (Re)$	× blue $(Re) (rE)$	Obtained... Theoretical.	35 34	33 34	0 0
6..	Black $(rE) (rE)$	× blue-splashed $(Re) (Re)$	Obtained... Theoretical.	0 0	138 138	0 0
7..	Blue-splashed $(Re) (Re)$	× black $(rE) (rE)$	Obtained... Theoretical.	0 0	56 56	0 0
8..	Blue-splashed $(Re) (Re)$	× blue-splashed $(Re) (Re)$	Obtained... Theoretical.	0 0	12 12	0 0

[2] Andalusians are normally homozygous for P, a factor necessary for the production of black pigment.

These results are in substantial accord with those of Bateson and Punnett (1906, p. 20). A somewhat marked departure from the theoretical expectation appears in group (3) of black ♂♂ × blue ♀♀ matings, the agreement in the reciprocal cross (group 2) being as close as possible. This departure from expectation is due to the progeny of a single pair of birds (♂136M and ♀2005) which produced 25 blues and 6 blacks. If the latter are left out of consideration the results are 88 blues and 84 blacks.

However, even in the case of the progeny of ♂136M and ♀2005 the Dev./P.E. = 4.1, which indicates a deviation of doubtful significance. The results of this mating were carefully considered from the standpoint of crossing-over, but there is no indication of its having occurred.

According to these results the genetic compositions of the three color types of Andalusians used in these experiments were as follows: blue-splashed $= (Re)(Re)$, blue $= (Re)(rE)$, and black $= (rE)(rE)$. There was no evidence of crossing-over between R and E having occurred.

VI. Data from Crosses of Andalusians with Certain Recessive White Breeds

In the previous paper (1918a, p. 106) the writer reported a small number of data on a cross between a white Wyandotte ♂ and a blue-splashed Andalusian ♀. These have been considerably increased in amount and the reciprocal cross made. Further, both blue and black Andalusians have been crossed reciprocally with white Wyandottes and all three Andalusian color types crossed reciprocally with white Plymouth Rocks. The data from these several matings are set forth in Table II.

The crosses were made in the twelve possible ways, from eleven of which offspring were secured, the one type of mating which failed to produce offspring being the white Wyandotte ♂ × black Andalusian ♀. Inasmuch as

there is no evidence that any of the factors here under observation are sex-linked and there is considerable evidence that they are not, this omission is not serious.

TABLE II

Showing the Results of Crossing the Three-color Types of Andalusians with White Wyandottes and White Plymouth Rocks

Group	♂ ♂	♀ ♀		Blue	Black
1.	Blue-splashed Andalusian × white Wyandotte		Obtained	65	00
	$PP(Re)(Re)$	$pp(rE)(rE)$	Theoretical	65	00
2.	White Wyandotte × blue-splashed Andalusian		Obtained	50	00
	$pp(rE)(rE)$	$PP(Re)(Re)$	Theoretical	50	00
3.	Blue-splashed Andalusian × white Plymouth Rock		Obtained	179	00
	$PP(Re)(Re)$	$pp(rE)(rE)$	Theoretical	179	00
4.	White Plymouth Rock × blue-splashed Andalusian		Obtained	87	00
	$pp(rE)(rE)$	$PP(Re)(Re)$	Theoretical	87	00
5.	Blue Andalusian × white Wyandotte		Obtained	27	24
	$PP(Re)(rE)$	$pp(rE)(rE)$	Theoretical	25.5	25.5
6.	White Wyandotte × blue Andalusian		Obtained	13	18
	$pp(rE)(rE)$	$PP(Re)(rE)$	Theoretical	15.5	15.5
7.	Blue Andalusian × white Plymouth Rock		Obtained	80	55
	$PP(Re)(rE)$	$pp(rE)(rE)$	Theoretical	67.5	67.5
8.	White Plymouth Rock × blue Andalusian		Obtained	24	32
	$pp(rE)(rE)$	$PP(Re)(rE)$	Theoretical	28	28
9.	Black Andalusian × white Wyandotte		Obtained	00	18
	$PP(rE)(rE)$	$pp(rE)(rE)$	Theoretical	00	18
10.	Black Andalusian × white Plymouth Rock		Obtained	00	132
	$PP(rE)(rE)$	$pp(rE)(rE)$	Theoretical	00	132
11.	White Plymouth Rock × black Andalusian		Obtained	00	28
	$pp(rE)(rE)$	$PP(rE)(rE)$	Theoretical	00	28

The results of these crosses are understandable on the assumption suggested in the earlier paper that the individuals from the recessive white races are homozygous for the factors E and p, p being the recessive allelomorph of P, a factor necessary for the production of black pigment in the feathers. Sturtevant (1912) first suggested that Wyandotte white is recessive, a fact which was overlooked in my earlier paper. Morgan and Goodale (1912, p. 115) have made a similar assumption for the white Plymouth Rock.

Since in the series of experiments being reported here, reciprocal crosses of white Wyandottes and white Plymouth Rocks gave only whites, thereby showing no

evidence of recombination, it seems fair to assume that the white of both breeds is due to the same recessive factor p in homozygous condition.

The condition of the white Rocks and white Wyandottes reported in Table II, with reference to E, appears clear, since in all crosses with blue-splashed Andalusians (and as will appear later, in the case of the Wyandotte, with blue-splashed Orpingtons) which are homozygous for P and R, but do not carry E, all offspring, 381 in number, were without an exception, blue (see mating groups 1 to 4, Table II).

On this basis blue Andalusians, $PP(Re)(rE)$, mated with such recessive whites should produce blues and blacks in equal numbers. Mating groups 5 to 8, inclusive, in Table II show the results of such matings, which combined give 144 blues to 129 black (136.5 to 136.5 would be equality), a fair realization of the expectation.

As would be expected from the foregoing, crosses of similar recessive whites with black Andalusians ($PP(rE)$ (rE)) (see Table II, groups 9 to 11, inclusive) gave only blacks. Of these there were in all 178 individuals and no exceptions.

The offspring of the crosses reported in Table II frequently gave evidence that the recessive white parents carried pattern factors as cryptomeres, but for the sake of clearness these complications, which have nothing directly to do with the study in hand, have been ignored in summarizing the data. As was to be expected, the white Plymouth Rocks carried the sex-linked pattern factor for barring. All pigmented offspring by a white Rock sire showed evidences of barring as soon as the definitive feathers appeared. Two such, the offspring of a white Plymouth Rock ♂ and blue Andalusian ♀ are shown in Fig. G, Plate II. Even at hatching, the occipital spot, which may be a juvenile effect of the factor for barring, gave notice of the presence of the barring factor. In the work here reported it was found possible to classify

in the down pigmented offspring of a non-barred ♂ × white Plymouth Rock ♀ cross accurately with regard to sex, by the presence or absence of the occipital spot. Morgan and Goodale (1912) made use of this spot in classifying barred and non-barred chicks which failed to hatch and Punnett (1919) also has made use of it in sorting newly hatched cross-bred chicks according to sex.

The progeny of crosses involving white Wyandottes frequently displayed Wyandotte lacing of a lesser or greater degree of perfection, though the appearance of this pattern was neither as constant nor as distinct as that of the barred pattern. The appearance of the lacing was to be expected if, as is generally stated in the literature on Wyandottes (see McGrew, 1901), the white variety was derived directly from the silver Wyandotte, which is laced.

In connection with these recessive white crosses is to be noted the fact that several white individuals, although " pure-bred " in the terminology of the poultryman, gave results which differed from the foregoing. Four white Wyandotte females proved to carry both the R and E factors and were of the same composition with respect to these factors as a pure-bred blue Andalusian, but unlike the blue Andalusian they carried p in the homozygous condition. One of these, which has already been reported on elsewhere (Lippincott, 1919), carried the sex-linked pattern factor for barring as well. Dryden (1916, p. 67) has also reported a white Wyandotte carrying a factor for barring.

One white Plymouth Rock and eight white Wyandottes proved to be heterozygous for a factor for dominant white. These were tested and found to be homozygous for p. In other words they carried both dominant and recessive white. Bateson and Punnett (1905, p. 117) appear to have had birds of this type and Dryden (1916, p. 66) reports a white Wyandotte which produced only white chicks when mated to a black Minorca, hence must

have been homozygous for a dominant white factor. Whether it carried P or p, the evidence does not show.

So far no attempt has been made to ascertain whether this factor for dominant white is the same as that normally carried by the white Leghorn and which Hadley (1913 and 1914) designated as I.   For convenience and to recognize the possibility of its differing from I the factor here dealt with is referred to in this paper as I^P (inhibitor of pigment) and its allelomorph as i^P.

VII. Back-crosses of F_1's from Blue-splashed Andalusian $\times$ Recessive White Matings

The results of crossing the F_1 blues from the blue-splashed Andalusian $\times$ recessive white crosses is shown in Table III.

While by no means all possible back-crosses have been made, enough are represented to show clearly that factors R and E were appearing in approximately equal numbers, and that this was also true of P and p, though in some cases the presence of I^P complicated matters somewhat. It was, unfortunately, not always possible to use the actual parents in making back-crosses and though individuals from the same families were employed, this proved to be no criterion that they would be of the same genotype as the individuals used in the original cross. There can be no question as to their factorial composition, however, as each individual has been either deliberately tested or had happened to be so mated for another purpose as to give dependable evidence on its composition with respect to I^P and p.

So far as it goes, the evidence, which is substantiated by the results of other crosses to be reported in a later section of this paper, also shows that the meeting of P and R was according to chance, thereby indicating no linkage between these two factors.

It will be noted that the blue F_1 ♀♀ in group 5 of Table III had a blue Andalusian mother instead of a blue-

TABLE III

SHOWING THE RESULTS OF BACK-CROSSING F₁ BLUES FROM BLUE AND BLUE-SPLASHED ANDALUSIAN × RECESSIVE WHITE CROSSES

Group	♂♂	♀♀		Blue-Splashed	Blue	Black	White
1...	Blue F₁ $\frac{\text{spl. And. ♂}^3}{\text{wh. Wyand. ♀}}$ $Pp(Re)(rE)$	× blue-splashed Andalusian $PP(Re)(Re)$	Obtained Theoretical	15 13.5	12 13.5	00 00	00 00
2...	Blue F₁ $\frac{\text{wh. Wyand. ♂}}{\text{spl. And. ♀}}$ $Pp(Re)(rE)$	× blue-splashed Andalusian $PP(Re)(Re)$	Obtained Theoretical	4 3.5	3 3.5	00 00	00 00
3...	Blue F₁ $\frac{\text{spl. And. ♂}}{\text{wh. Rock ♀}}$ $Pp(Re)(rE)$	× blue-splashed Andalusian $PP(Re)(Re)$	Obtained Theoretical	25 24.5	24 24.5	00 00	00 00
4...	Blue F₁ $\frac{\text{wh. Wyand. ♂}}{\text{spl. And. ♀}}$ $Ppi_vP'P(rE)(rE)$	× white Wyandotte $ppI_vP'_vP(rE)(rE)$	Obtained Theoretical	00 00	12 10.375	7 10.375	64 62.250
5...	White Plymouth Rock × blue F₁ $\frac{\text{blue And. ♀}}{\text{wh. Wyand. ♂}}$ $pp(rE)(rE)$	$Pp(Re)(rE)$	Obtained Theoretical	00 00	8 10	8 10	24 20
6...	White Wyandotte × blue F₁ $\frac{\text{spl. And. ♂}}{\text{wh. Wyand. ♀}}$ $ppI_vP'_vP(rE)(rE)$	$Ppi_vP'(Re)(rE)$	Obtained Theoretical	00 00	3 5.375	7 5.375	33 31.250

³ This convention in this and subsequent tables is used to indicate the kind and direction of the original cross.

splashed. From the nature of the behavior of the factors
R and E already described, this would make no difference
with regard to the blue offspring, for the blue progeny of
a blue Andalusian female by a white Wyandotte male
would be of exactly the same composition with respect
to R, E, and P as *all* the offspring of a blue-splashed
Andalusian mother by the same sire.

It will also be noted in this group (5) that while the
father of the F_1 blue was a white Wyandotte, the male
used in this cross was a white Plymouth Rock. Since it
has been shown that for the factors being studied, white
Plymouth Rocks and white Wyandottes are identical, this
should not affect the ratios.

VIII. The F_2 Ratios from Blue-splashed Andalusian
$\times$ Recessive White Matings

The F_2 ratios from various blue-splashed Andalusian
$\times$ recessive white crosses are shown in Table IV.

As will be seen, the four F_2 classes predicted for
such crosses in the writer's earlier paper (1918*a*, p. 113)
on the basis of the F_1 results, have been obtained. No
other classes have appeared. This would seem to indicate
that the factorial compositions of the blue-splashed
Andalusians and white Wyandottes then proposed were
correct and that the white Plymouth Rocks used were of
the same composition with respect to the factors R, E
and P as were the white Wyandottes.

Seven F_1 blue males were used in securing the F_2
ratios. The legband numbers of these males may be
found in Table IV, in the column headed "Band No."
The direction of the original cross is indicated for each
male and for the group of females with which he was
mated. The direction of the cross was the same for the
males and the females in all cases but two. Males 296M
and 258M were mated with females which were products
of the same crosses, respectively, as they themselves
(groups 2 and 7), and also with females from the recip-
rocal crosses (groups 3 and 8).

TABLE IV

SHOWING F_2 RATIOS FROM CROSSES OF BLUE-SPLASHED ANDALUSIANS AND WHITE WYANDOTTES AND WHITE PLYMOUTH ROCKS[4]

Group	♂	Band No.	♀♀		Blue	Blue-spl.	Black	White
1...	Blue F_1 $\dfrac{\text{spl. And. ♂}}{\text{wh. Wyand. ♀}}$ $X^2 = 4.5747$	$\dfrac{86E \times \text{blue } F_1}{}$ $P = .2099$	$\dfrac{\text{spl. And. ♂}}{\text{wh. Wyand. ♀}}$	Obtained	100	46	65	64
				Theoretical	103.1250	51.5625	51.5625	68.7500
2...	Blue F_1 $\dfrac{\text{spl. And. ♂}}{\text{wh. Wyand. ♀}}$ $X^2 = 4.0998$	$\dfrac{296M \times \text{blue } F_1}{}$ $P = .252516$	$\dfrac{\text{spl. And. ♂}}{\text{wh. Wyand. ♀}}$	Obtained	73	24	39	48
				Theoretical	69	34.5	34.5	46
3...	Blue F_1 $\dfrac{\text{spl. And. ♂}}{\text{wh. Wyand. ♀}}$ $X^2 = .8759$	$\dfrac{296M \times \text{blue } F_1}{}$ $P = .83592$	$\dfrac{\text{wh. Wyand. ♂}}{\text{spl. And. ♀}}$	Obtained	45	17	20	27
				Theoretical	40.875	20.4375	20.4375	27.2500
4...	Blue F_1 $\dfrac{\text{wh. Wyand. ♂}}{\text{spl. And. ♀}}$ $X^2 = 5.7301$	$\dfrac{66M \times \text{blue } F_1}{}$ $P = .1278$	$\dfrac{\text{wh. Wyand. ♂}}{\text{spl. And. ♀}}$	Obtained	19	14	7	6
				Theoretical	17.25	8.625	8.625	11.5
5...	Blue F_1 $\dfrac{\text{wh. Wyand. ♂}}{\text{spl. And. ♀}}$ $X^2 = 4.6871$	$\dfrac{65E \times \text{blue } F_1}{}$ $P = .1998$	$\dfrac{\text{wh. Wyand. ♂}}{\text{spl. And. ♀}}$	Obtained	94	41	44	42
				Theoretical	82.875	41.4375	41.4375	55.2500
6...	Blue F_1 $\dfrac{\text{spl. And. ♂}}{\text{wh. Rock ♀}}$ $X^2 = 2.7349$	$\dfrac{46E \times \text{blue } F_1}{}$ $P = .4395$	$\dfrac{\text{spl. And. ♂}}{\text{wh. Rock ♀}}$	Obtained	34	10	17	18
				Theoretical	29.625	14.8125	14.8125	19.75
7...	Blue F_1 $\dfrac{\text{spl. And. ♂}}{\text{wh. Rock ♀}}$ $X^2 = 6.7510$	$\dfrac{258M \times \text{blue } F_1}{}$ $P = .081786$	$\dfrac{\text{spl. And. ♂}}{\text{wh. Rock ♀}}$	Obtained	51	26	39	51
				Theoretical	62.625	31.3125	31.3125	41.75
8...	Blue F_1 $\dfrac{\text{spl. And. ♂}}{\text{wh. Rock ♀}}$ $X^2 = 8.20$	$\dfrac{258M \times \text{blue } F_1}{}$ $P = .042668$	$\dfrac{\text{wh. Rock ♂}}{\text{spl. And. ♀}}$	Obtained	14	3	3	1
				Theoretical	7.8750	3.9375	3.9375	5.25
	Total ratios for all crosses $X^2 = 8.6410$ $P = .0353$			Obtained	430	181	234	257
				Theoretical	413.25	206.625	206.625	275.500

[4] The formulæ of color types involved in these crosses are: blue-splashed Andalusian PP (Re) (rE), white Rocks and Wyandotes pp $(rE)(rE)$, and F_1 blues $Pp(Re)(rE)$.

As may be seen by inspection of Table IV, but one male (296M) gave a group of offspring (3) which was very close to expectation. The chances that as great a deviation as this one would appear as a result of random sampling are four to one. The mothers of this group were the product of a cross which was the reciprocal of that which produced their sire. The offspring of 296M when mated with females which were the product of the same cross as himself (group 2) gave a deviation so great that the chances against its appearing as a result of random sampling are three to one. The chances of the appearance of deviations as great as those shown by the offspring groups of the other males were as follows: 86E (group 1) one chance in a little less than five; 66M (group 4) one chance in about eight; 65E (group 5) one chance in approximately five; 46E (group 6) one chance in about two and a quarter; 258M (group 7) once in about twelve times with females from the same cross as he, and once in twenty-five when mated with females from a reciprocal cross (group 8).

It would be unusual, though not impossible, to have so many comparatively wide deviations from expectation simply as a result of random sampling.

If the genetic constitution of the F_1's was as has been previously postulated, and these were in fact all chance deviations, it would be highly probable that the lumping of all the data given in Table IV would approximate the calculated ratio fairly closely.

The lumped data are given at the bottom of Table IV. It will at once be seen that the goodness of fit as measured by P is poorer than the poorest constituent group, and would be probable, on the basis of random sampling, once in about twenty-eight times. It seems fairly clear that some disturbing force was operative.

The two possible causes of disturbance which present themselves are linkage and a differential viability of classes, or it might be a combination of the two.

Linkage between the two principal pairs of factors involved in the crosses, Pp and $(Re)(rE)$, may not be appealed to because the only possible linkage relation would produce results diametrically opposed to those with which we are confronted. Since according to our hypothesis the recessive white parents were in each case of the composition $pp(rE)(rE)$ and the blue-splashed Andalusian parent $PP(Re)(Re)$, it is evident that linkage would require the production of $p(rE)$ gametes by the F_1 blues, more often than $P(rE)$ gametes. And similarly the combination $P(Re)$ should also appear more often than $p(Re)$.

A complete linkage between these pairs of allelomorphs would result in an F_2 ratio of 1 blue-splashed and 2 blue to 1 white, the blacks not appearing. The tendency of even weak linkage would be to reduce the proportional number of blacks. This should be true irrespective of the direction of the cross. It would further be true, that unless crossing-over occurred in both sexes any linkage whatsoever would inhibit the production of F_2 blacks homozygous for P. As will be shown in a later section of this paper, however, F_2 blacks homozygous for P have been identified. Even a casual inspection of Table IV shows that a relative preponderance of blacks is a quite constant characteristic.

Crossing-over in the male fowl has been found by Goodale (1917) and in the male pigeon by Cole and Kelley (1919). The latter investigators definitely state that there is no crossing-over of sex-linked factors in the female pigeon. Goodale states that none had been observed in the female fowl, but that a definite test of the matter would be made later. So far as the writer is aware no further report has been made. It should perhaps be pointed out that so far only sex-linked factors have been dealt with, no autosomal linked groups in birds having so far been reported.

There are no F_2 data available from crosses where p and (Re) are found in one parent and P and (rE) in the

other.   The F_1's from such a cross have been secured by mating an extracted white of the composition $pp(Re)(Re)$ with a black Andalusian, $PP(rE)(rE)$, which gave all blues.   From these an attempt will be made to secure F_2's in considerable numbers.   Back-crosses to the parental types will also be made.   The F_2's should approximate the same ratios as appear in Table IV and also give some evidence on the second possible explanation of the persistent deviations about to be discussed.

The calculation of theoretical expectancies presupposes the equal viability of all phenotypic and genotypic classes.   If for any reason the individuals of one or more of the obtained classes tend to be less viable than certain other classes, deviation from expectancy will occur if the lack of viability expresses itself prior to making the counts.

As has already been pointed out, the lumping of the data presented in Table IV brings forth a poorer fit than is shown in any of the constituent groups.   The deficient classes are the blue-splashed and the white, while the most preponderant class relatively is the black.

It seems to be a rather tacit assumption among poultrymen, particularly, it must in truth be said, among those breeding pigmented varieties, that the recessive white varieties are less vigorous (and so in all probability less viable) than the pigmented varieties of the same breeds. In how far this assumption is based on fact there is no critical evidence to call upon.

Regarding the relative viability of splashed and self-colored races there is no suggestion from any source. Splashed varieties are, so far as I am aware, nowhere bred as such, and the experience of practical breeders may accordingly not be appealed to.

While in the case in hand the assumption of low viability on the part of the individuals of the splashed and recessive white classes seems to correspond with the facts, such an assumption, though convenient, is not cor-

roborated by other evidence. That the splashed classes are not necessarily always deficient is shown by the progeny of the blue-splashed × blue mating in Table I, group 5, and of the F_1 blue × blue-splashed matings in Table III, groups 1, 2 and 3.

The latter fact suggests that possibly certain individuals used in these matings carried recessive factors tending to cause low viability, which were linked to the factor R. Until the fact of a differential viability is demonstrated, however, it is useless to speculate on this possibility. The reason for the deficiencies in the blue-splashed and also in the white classes, therefore can not at present be determined.

IX. IDENTIFICATION OF THE F_2 GENOTYPES

As indicated in my former paper (1918*a*, p. 113) the genotypes expected in the several F_2 phenotypes from the blue-splashed × recessive white crosses are as follows: blue, $PP(Re)(rE)$ and $Pp(Re)(rE)$; blue-splashed, $PP(Re)(Re)$ and $Pp(Re)(Re)$; black, $PP(rE)(rE)$ and $Pp(rE)(rE)$; white, $pp(Re)(Re)$, $pp(Re)(rE)$ and $pp(rE)(rE)$. Although the limitations of equipment were such that comparatively few F_2 individuals could be tested, fortunately all of the genotypes but one have been identified by making the appropriate crosses. The blues mated to individuals homozygous for p and E gave blues and blacks in equal numbers, or, blues, blacks and whites in the approximate ratio of $1:1:2$, as the case might be. The blue-splashed mated to individuals of the same constitution produced all blues, or, equal numbers of blues and whites, depending upon whether or not they were homozygous with respect to P. Similarly the blacks gave all blacks, or, blacks and whites, depending upon their condition with respect to P.

The whites on the other hand were mated to blacks known to be homozygous for P and E. The $pp(Re)(Re)$ whites, as mentioned in an earlier section of this paper,

gave all blues, just as would blue-splashed Andalusians. The $pp(Re)(rE)$ whites produced blacks and blues in approximately equal numbers, exactly as would blue Andalusians. The parental white genotype $pp(rE)(rE)$, which would give all blacks, was curiously enough, the one of the whites which did not happen to be selected for testing.

It is important to note that while eight out of the nine F_2 genotypes were identified, no genotypes were found other than those expected.

X.   DATA ON ANDALUSIAN $\times$ BLACK LANGSHAN CROSSES

It appeared desirable, in order to ascertain whether there was anything inherent in Andalusian black which made its relation to Andalusian blue different from that of other black breeds, to make certain matings of Andalusians with black Langshans. The Langshan was chosen because not only is it a different breed, but it also belongs to a different group of breeds. The original black Langshans were, according to Brown (1906, p. 63), imported from China, while the Andalusians, according to the same authority (p. 107), originated from native stocks along the borders of the Mediterranean Sea. So far as is known they have nothing in common in their immediate ancestry. Davenport (1914) even points to the probability that the immediate wild ancestors of the Asiatic breeds differed from those of the Mediterranean breeds. If blacks differ in their relation to Andalusian blue it would seem probable that Andalusian black and Langshan black might show this difference.

The results of the Andalusian-Langshan matings are shown in Table V. As may be seen readily by reference to this table the results are in every case precisely those which might be expected if a black Andalusian had been substituted for the black Langshan. So far as the principal factors under discussion are concerned it appears that the black Langshans used were identical in composi-

TABLE V

Group	♂	♀ ♀		Blue Splashed	Blue	Black
1..	Blue Andalusian $PP(Re)(rE)$	× black Langshan $PP(rE)(rE)$	Obtained Theoretical	0 0	34 32.50	31 32.50
2..	Blue Andalusian $PP(Re)(rE)$	× black $\dfrac{\text{wh. Wyand.♂}}{\text{black Lang. ♀}}$ $Pp(rE)(rE)$	Obtained Theoretical	0 0	15 18	21 18
3..	Blue-splashed Andalusian $PP(Re)(Re)$	× black Langshan $PP(rE)(rE)$	Obtained Theoretical	0 0	65 65	0 0
4..	Black Andalusian $PP(rE)(rE)$	× black Langshan $PP(rE)(rE)$	Obtained Theoretical	0 0	0 0	11 11
5..	Blue $\dfrac{\text{blue And. ♂}}{\text{black Lang. ♀}}$ $PP(Re)(rE)$	× blue $\dfrac{\text{blue And. ♂}}{\text{black Lang. ♀}}$ $PP(Re)(rE)$	Obtained Theoretical	4 4.5	9 9	5 4.5
6..	Blue $\dfrac{\text{blue And. ♂}}{\text{black Lang. ♀}}$ $PP(Re)(rE)$	× black $\dfrac{\text{blue And. ♂}}{\text{black Lang. ♀}}$ $PP(rE)(rE)$	Obtained Theoretical	0 0	12 10.5	9 10.5
7..	Blue $\dfrac{\text{blue And. ♂}}{\text{black Lang. ♀}}$ $PP(Re)(rE)$	× black Langshan $PP(rE)(rE)$	Obtained Theoretical	0 0	12 12.5	13 12.5
8..	Blue $\dfrac{\text{blue And. ♂}}{\text{black Lang. ♀}}$ $PP(Re)(rE)$	× blue Andalusian $PP(Re)(rE)$	Obtained Theoretical	5 3.25	6 6.5	2 3.25

tion with the black Andalusians, being $PP(rE)(rE)$. The condition of the Langshan with respect to P was found by mating individuals with white Wyandottes, whereby only black, *i.e.*, pigmented, offspring were produced.

XI. THE RELATION OF ORPINGTON BLUE TO ANDALUSIAN BLUE

Among the Orpingtons, an English breed, is a blue variety. Like the blue Andalusian it is an inconstant breeder with regard to color, segregating into blue-splashed and blacks as well as blues. Though by no means as widely bred as the blue Andalusians, it has numerous admirers, some of whom have claimed verbally to the writer that the proportion of wasters, *i.e.*, blue-splashed and blacks, was much smaller than in the Andalusians, though no figures are obtainable by way of sub-

TABLE VI

SHOWING THE RESULTS OF CERTAIN BLUE, BLUE-SPLASHED AND BLACK ORPINGTON CROSSES AMONG THEMSELVES AND WITH OTHER BREEDS

Group	♂	♀♀		Blue-spl.	Blue	Black	White
1...	Blue Andalusian (Re)(rE)	× blue Orpington (Re)(rE)	Obtained	13	30	12	0
			Theoretical	13.75	27.50	13.75	0
2...	Blue-splashed Andalusian (Re)(Re)	× black Orpington (rE)(rE)	Obtained	0	37	0	0
			Theoretical	0	37	0	0
3...	Black Andalusian (rE)(rE)	× blue-splashed Orpington (Re)(Re)	Obtained	0	14	0	0
			Theoretica	0	14	0	0
4...	White Wyandotte pp(rE)(rE)	× blue-splashed Orpington PP(Re)(Re)	Obtained	0	21	0	0
			Theoretical	0	21	0	0
5...	Blue Orpington (Re)(rE)	× blue Orpington (Re)(rE)	Obtained	19	58	15	0
			Theoretical	23	46	23	0
6...	Blue F$_1$ spl. Orp. $\frac{\text{wh. Wyand. ♂}}{Pp(Re)(rE)}$	× blue F$_1$ spl. Orp. $\frac{\text{wh. Wyand. ♀}}{Pp(Re)(rE)}$	Obtained	60	26	24	25
			Theoretical	50.6250	25.3125	25.3125	33.7500

$X^2 = 4.0505$ $P = .2569$

stantiation. It seemed desirable from several standpoints to ascertain what factors were involved in the production of Orpington blue, and whether the blue Orpington differed from the blue Andalusian in its genetic behavior. A number of matings were accordingly made, the data from which are shown in Table VI.

These data are consistent with the supposition that the factors involved in the production of Orpington blue are identical with those which produce Andalusian blue. The crossing of blue Andalusians and blue Orpingtons gave exactly the same sort of result as that obtained by mating blue Andalusians *inter se,* as shown by group 1. The blue-splashed Orpingtons mated with white Wyandottes gave only blues (group 4) just as did the blue-splashed Andalusians. And finally the F_2 ratio from white Wyandotte $\times$ blue-splashed Orpington crosses gave the same phenotypic classes as were obtained in the F_2 from the white Wyandotte $\times$ blue-splashed Andalusian cross, with a deviation from expectancy as great as would be probable once in four times. It is interesting to note that while the white class is deficient in this case, the blue-splashed class is not.

XII. Data from Blue Leghorn Crosses

In the spring of 1917 there appeared in the large pure-bred single comb white Leghorn flock of the Pabst Stock Farm at Oconomowoc, Wisconsin, two blue females. The flock was not pedigreed and nothing is known of the individual ancestors of these birds. They were of fair Leghorn type and were, as far as known, the offspring of pure-bred white Leghorn parents. Through the courtesy of Mr. Fred Pabst, and Dr. L. J. Cole of the University of Wisconsin, these individuals came into the hands of the writer and were entered on the records of the Department of Poultry Husbandry of the Kansas State Agricultural College as numbers 767 and 768.

Number 767 was a fairly even shade of medium to light

blue when received and showed some evidence of barring, though this was not very distinct. Number 768 was much lighter in shade than 767 and showed no evidence of barring. In contrast with ordinary blue she would, from a little distance, be mistaken for a white. The pigment granules in both cases were round.

The results of mating these birds in various ways are presented in Table VII. The numbers are rather small

TABLE VII

SHOWING THE BREEDING BEHAVIOR OF TWO BLUE LEGHORN FEMALES, WHEN MATED WITH VARIOUS MALES OF KNOWN FACTORIAL COMPOSITION

♂	♀		Blue Splashed	Blue	Black	White
White Leghorn 117M $IIPP(rE)(rE)$	× 767 $iiPP(Re)(rE)$	Obtained	0	0	0	5
		Theoretical[5]	0	0	0	5
White Leghorn 117M $IIPP(rE)(rE)$	× 768 $iiPP(Re)(rE)$	Obtained	0	0	0	11
		Theoretical	0	0	0	11
Blue Andalusian 78M $PP(Re)(rE)$	× 767 $PP(Re)(rE)$	Obtained	2	4	2	0
		Theoretical	2	4	2	0
Blue Andalusian 78M $PP(Re)(rE)$	× 768 $PP(Re)(rE)$	Obtained	3	7	4	0
		Theoretical	3.5	7	3.5	0
White Plymouth Rock 155M $pp(rE)(rE)$	× 767 $PP(Re)(rE)$	Obtained	0	20	24	0
		Theoretical	0	22	22	0
White Wyandotte 192M $I^Pi^Ppp(rE)(rE)$	× 768 $i^Pi^PPP(Re)(rE)$	Obtained	0	8	8	12
		Theoretical	0	7	7	14
Blue white Rock ♂155M 255M blue Leghorn ♀ 767 $Pp(Re)(rE)$	× 767 $PP(Re)(rE)$	Obtained	2	6	1	0
		Theoretical	2.25	4.50	2.25	0
Black Andalusian 288M $PP(rE)(rE)$	× 768 $PP(Re)(rE)$	Obtained	0	25	22	0
		Theoretical	0	23.5	23.5	0

but two facts seem fairly evident. First, that 767 and 768 are alike with respect to the factors under discussion in this paper, and second, that they give no indication of being different in their make-up with respect to the factors R, E and P from pure-bred blue Andalusians.

The appearance of the blue offspring of 768 (which it will be recalled was very light) when mated with black or blue Andalusians, was such as to suggest the possibility that accessory factors, necessary for the production of blue of normal shade, were supplied by the Anda-

[5] The theoretical expectancies calculated as for blue Andalusians.

lusian males, though no attempt was made to isolate and identify them.

Since these blue Leghorns arose in an unpedigreed flock, their origin is conjectural. A plausible explanation seems to be that two individuals heterozygous for *I,* the dominant Leghorn factor described by Hadley (1913), which inhibits the production of pigment shown (also by Hadley, 1914) to be normally present in the white Leghorn, happened to mate and that at least one of them carried the factor *R* as a cryptomere. That white Leghorns may sometimes carry the factor seems to be shown by the fact that Dryden (1916, p. 67) secured blue chicks in an F_2 generation from a barred Plymouth Rock $\times$ white Leghorn cross. And further, in the course of the breeding operations reported in this paper, blues appeared in the progeny of a black Andalusian and a white crossbred, the latter being the product of a black Andalusian $\times$ white Leghorn cross. In both cases it appears that the factor *R* must have been brought in by the white Leghorn. This suggestion also involves the assumption that the white Leghorn carries the factor *E*. That this is the case is shown by the fact that in the F_2 from a blue-splashed Andalusian $\times$ white Leghorn cross, the details of which are reserved for later publication, both blacks and blues appeared.

XIII. The Problem of True-breeding Blues

The fact that the blue varieties of both Andalusians and Orpingtons as now constituted do not breed true is a matter of considerable importance to their breeders. It is a heavy handicap to both varieties. While one hundred per cent. of blues may in each case be secured by mating blue-splashed individuals with black, as a matter of practical breeding this mating is seldom made. This is owing to the fact that there are several more or less variable qualities of color for which rigid selection is practised which are not apparent in either the blue-

splashed or blacks.   The breeder therefore prefers to use for breeding purposes only those individuals which show the desired phenotypic condition, even though so doing necessitates the discarding of approximately half the offspring.   While this leaves a comparatively small number of individuals, as compared with other breeds, upon which to practise selection, the blue Andalusian at least is bred in considerable numbers, thereby indicating its economic desirability and its attractiveness.

As was pointed out in the earlier paper (1918, p. 111) if R and E are not at identical loci on homologous chromosomes and crossover individuals were found which produce RE gametes, the problem of the constant-breeding blue would be solved.

The situation regarding black in rats may not be without its bearing in the present case.   Black rats which bred true have been known for some time.   Castle (1919) has, however, reported certain races of blacks which failed to breed true.   This type of black was tested through several generations by Castle (1919), Ibsen (1920) and Dunn (1920).   Blacks mated to blacks quite persistently produced whites, blacks and red-eyed yellows in the ratio of 1 to 2 to 1.   Castle (1919) found one possible cross-over individual which died without being tested.   Ibsen (1920) has so far failed to find any, and Dunn (1920) reports between one and two per cent. of cross-overs.   These cross-overs, which were longer sought for and among larger numbers than has yet been possible with Andalusians, would appear to make it possible to synthesize a true breeding (*i.e.*, homozygous) black, from the line which has not been breeding true through a considerable number of generations.

It is also worth noting in this connection the possible bearing of Sturtevant's (1919) finding families of *Drosophila* carrying at least two definite factors in the second chromosome which almost completely inhibit crossing-over in the region contiguous to their loci.

If, however, after a long-continued search, it becomes increasingly evident that R and E are indeed allelomorphs, as originally suggested by Bateson and Punnett (1905), it was suggested (p. 113) that hope might be seen in the progressive selection of the darker, that is, more fully pigmented, blue-splashed individuals, there being considerable variation among the latter in this regard.

There is a further possibility which should not be overlooked, namely, that other factors might be found, perhaps in other breeds, which would act on black pigment to give the blue appearance on the one hand, or extend it to give self-colored individuals on the other. If duplicate factors for E or R should be found, a means of producing the long sought true-breeding blue would seem to be at hand. The fact that three factors are known which produce white in fowls lends emphasis to the possibility. It would seemingly make little difference in the ultimate outcome whether the new factor was linked to R and E, or was located on a different chromosome pair. In either case it would be possible to get a ''self-coloring'' and a ''bluing'' factor in the same gamete which, it appears, has so far not been done.

XIV. SUMMARY

1. It has been shown that the development of black pigment in the blue-splashed, blue and black races of the Andalusian and Orpington breeds, and of black Langshans, depends upon the action of a dominant hereditary factor P, for which they are normally homozygous.

2. The allelomorph of P is p. Individuals homozygous for p are white, as in the white Wyandotte and white Plymouth Rock breeds.

3. The extension of black pigment to all feathers of the body, resulting, if no pattern factors are present, in self-colored individuals, depends upon a dominant factor E. This factor has been found in the Andalusian, Orpington, white Plymouth Rock, white Wyandotte and black Lang-

shan breeds. Some evidence is presented which indicates its presence in white Leghorns.

4. The blue appearance of blue and blue-splashed Andalusians and Orpingtons, is due to the arrangement and restriction of black pigment, the result of a dominant factor R. This factor has also been found in individuals of the white Wyandotte and white Leghorn breeds, though its presence is probably not usual in these breeds.

5. No individuals of the Andalusian, Orpington, white Plymouth Rock, white Wyandotte or black Langshan breeds have been found which did not carry R, E or both.

6. The mutual relations of R and E are such that they have never been found together in the same gamete. This indicates that they are allelomorphic, *i.e.*, occupy identical loci on homologous chromosomes, or, each is so closely linked to the recessive allelomorph of the other, (Re) and (rE), that crossing-over rarely, if ever, occurs.

7. No evidence of crossing-over between R and E has been found and the tentative conclusion must be in accord with that previously held, that R and E are allelomorphs.

8. Both R and E are independent of P in their hereditary behavior, though dependent upon its presence for their manifestation.

9. The cooperative influence of the ovary is necessary for a full expression of R in the regions of the neck, back and saddle.

10. On the basis of the evidence presented in the body of this paper the genetic formulæ of the breeds and varieties employed, with respect to the factors under observation, are usually as follows: blue-splashed Andalusians and Orpingtons $PP(Re)(Re)$; blue Andalusians and Orpingtons $PP(Re)(rE)$; black Andalusians, Orpingtons and Langshans $PP(rE)(rE)$; and white Plymouth Rocks and Wyandottes $pp(rE)(rE)$.

11. The possibility of the occurrence of factors which duplicate the somatic effects of R and E is pointed out, and the relation of this possibility to the production of constant-breeding blues briefly discussed.

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